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Barks and leaves of Lauraceae plants as anti-acne

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Abstract: *Acne vulgaris* is an inflammatory skin disease caused by several factors, including an imbalance of microorganisms compared to the normal microbial distribution in healthy tissue. The *Lauraceae* family which have been reported from antiseptic. This research was conducted to assayed the compounds, the biological activity of the *Lauraceae* plant essential oil (EO) could against microbes. EO Were isolated by steam water distillation and had antibacterial effects were analyzed by microdilution in broth medium and EO compounds were analyzed by GC-MS. The most abundant components present in *N. cassia* leaves cinnamyl acetate, *N. cassia* bark beta-citronellol, linalool, e-citral and geraniol, *C. verum* bark eugenol, *C. burmanni* leaves linalool, alpha-terpineol, eucalyptol, cinnamaldehyde, caryophyllene, cinnamyl, leaves of *Cinnamomum camphora* contains camphor. The highest antimicrobial activity against *C. acnes* was given by oil from the leaves and bark of *Neolitsea cassia*, against *S.aureus* and by oil from the bark of *C. verum* and *N. cassia* and *C. burmannii* leaves. The oil of *C. champora* leaves gave better activity than the barks against these microbes. It was found that the leaves of *C. champora* had the highest yield of oil compared to the other part of *Lauraceae* plants tested and it has a strong antibacterial activity toward microbes commonly present in acne vulgaris.

Keywords: GCMS spectrum, antimicrobial, *Cinnamomum species*, EO.

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INTRODUCTION

Lauraceae is one of the most primitive families of plants, that resulted essential oil. Acne is maximum normally because of *Propionibacterium acnes*, however *Staphylococcus aureus* additionally reasons persistent irritation of the furry base of the face, neck and higher body (Achermann *et al*., 2014). Factors that contribute to the development of acne include increased sebum production, keratinization of the ducts, bacterial invasion, and inflammation of the cystic ducts. Although the severity of acne vulgaris is related to sebum secrection, the disease is one of the cystic funnels. With a mild form of acne, keratinocytes in the funnel become hyper keratinized and hypo desquamate forms comedones. In severe acne, the funnel ruptures and sebum enters the dermis, causing severe inflammation (Jappe, 2003). Acne treatment includes topical and systemic therapies (Achermann *et al*., 2014). However, long-term excessive use of antibiotics can increase the bacterial resistance of acne (Patel *et al*., 2010). To overcome antibiotic resistance, essential oils provide safer, more effective and versatile alternative solutions. Previous studies have shown that Lauraceae has the potential activity against bacteria (LeBel *et al*., 2017). There is a close relationship between the assymetry of *Lauraceae* organic compounds and their bioactivity (Damasceno *et al*., 2019). The aim of this study were to known the most abundant components in *Lauraceae*, critism the antimicrobial activity of EO from the barks also the leaves of *Lauraceae* plants against the bacteria caused acne.

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MATERIALS AND METHODS

Sample collection

Approximately 1kg of leaves and barks of five Lauraceae plants namely *C. burmanni, C. camphora, C. sintoc, C verum*, and *Neplitsea* were collected from the botanic garden of Cibinong, Bogor, West Java, Indonesia Bogoriense with number of letter B-403/IV/DI.01/3/2021. Fresh samples were cut into pieces for the isolation of their volatile oils through steam distillation.

Lauraceae samples were collected up to 1kg, consisting of two parts of the bark and leaves of each of the five Lauraceae plants (*C. burmanni* (Ness & Nees) Blume*, C. champora* (L) J. Presl.*, C. sintoc* Blume*, C. verum,* J. Presl. and *Neolitsea cassia* (L.) Kosterm. The barks and leaves were taken in the afternoon of the same day. Samples were cut for steam distillation.

Taxonomic determination

Lauraceae samples were determined at the Indonesian Institute of Sciences in Bogor, Indonesia.

The samples were washed and then steam distilled. The essential oil was isolated from one kg of the fresh stem barks and the fresh leaves. Stem barks and the leaves of each plant part were cut into small pieces and placed in the upper distillation chamber. Water was poured into the lower chamber, until reaching the zero mark. Then, a vacuum was connected to the distillation chamber. The light was turned on at a low temperature to avoid the decomposition of oil due to its thermal stability. The essential oil distillate was collected using a clean vial. The distillation process was done for 5 hours.

Oil component analysis

Analysis of all the *Lauraceae* EO were performed using GC/MS analysis (Shimadzu GC/MS-QP 2010 Ultra®). The GC system in stationary phase: Rtx-5MS; mobile phase: helium ultra-high white; column pressure: 53.5 kPa; injection volume: 1µl, injector temperature 250°C, ion source temperature 250°C, interface temperature 230°C, split mode 100:1. The column is programmed from 50°C held for 5 minutes and then raised to 150°C at an increasing rate of 5°C/min until maximum temperature of the column was 300°C with an optimal rate of 10°C/min.

Preparation of MHB and MHA

Preparation of Mueller Hinton Broth (MHB), Mueller Hinton Agar (MHA) (https://himedialabs.com/TD/M173.pdf; https://himedialabs.com/TD/M391.pdf).

In this experiment, three tested microbe strains were chosen from the American Type Culture Collection (ATCC), which were *Staphylococcus aureus* (SA) ATCC® 6538, *Staphylococcus epidermidis* (SE) ATCC® 12228 and *Propionibacterium acnes* (Cutibacterium acnes/CA) ATCC® 11827 obtained from LIPI and cultured in the microbiology laboratory of the School of Pharmacy, Bandung Institute of Technology. SA, SE and CA can grow well in MHA and MHB. The medium was prepared by dispersing 34g of MHA and 21g of MHB in powder in 1,000mL water. Then, it was heated to yield a clear solution. The prepared medium was then sterilized using an autoclave at 121°C for 15 minutes.

Sterilization of materials and apparatus

Apparatus, agar, broth, and water were sterilized using an autoclave at 121°C for 15 minutes. The sterilized device then dried in an oven at 40°C to remove residual water.

Preparation of suspended microorganism

Agar slant was streaked using an oose needle with the microbe. Then, it was incubated at 35°C for 24 hours. Then, one Oose of microbe from agar slant was transferred into 10mL of suitable broth and incubated. The suspended microbe was diluted the next day until it reached 0.5 Mc. Farland standard, or the UV-Vis spectrophotometer absorbance range from 0.08 to 0.13. Then, dilution was done again with broth to a ratio of 1:20.

Determination of total colony forming unit (CFU)

One milliliter of microbe suspension was mixed homogenously with 20mL of suitable agar for several dilutions. Then the agar was incubated for 24 hours. The colony formed was counted by a Colony Counter. The initial bacteria suspension (stock) concentration was calculated from a petri dish containing 30-300 CFU (CLSI Method, 2009).

Minimum inhibitory concentration (MIC) determination

The micro dilution method was used to determine the MIC of oils obtained from five Lauraceae plants. In this method, a micro dilution plate with 96 wells was used. Firstly, all wells were added with 100µl of broth. Then, column number 12 was filled with oil diluted with DMSO 10% and then dilution was done transferring 100µl from column number 12 to number 3. Then, 100µl was discarded from column number 3. Finally, 10µl of microbe was added from columns numbers 2 to 12. The same steps were repeated for all samples. The micro dilution plate was incubated at 35°C for 18-24 hours (CLSI Methods, 2009).

RESULTS

GC-MS analysis

The yields of essential oils from the barks and leaves by using hydro distillation are presented in table 1. The EO from the leaves of *C. camphora* was presented as solid crystals while the other samples were liquid. *C. sintoc* and *N. cassia* barks and leaves contained very low volatile ranging from 0.01 to 0.05 only. The highest oil content was *C. camphora* leaves which yielded 1.5% oil from the fresh sample. These results are in line with the results of previous studies on the essential oil from the barks which yielded 0.5-5% and the leaves yielded 0.5-1.2% (Krishnamoorthy *et al*., 1999; Wang *et al*., 2009; Hasanah *et al*., 2003; Xu *et al*., 2004; Geng *et al*., 2011; Yuliarto *et al*., 2021).

Table 1: The yield of essential oils from five Lauraceae plants

No	Sample	Bark (%)	Leaf (%)
1.	*C. burmanni* (Ness & Nees) Blume	0.1	0.13
2.	*C. sintoc* Blume	0.02	0.01
3.	*C. verum* J. Presl.	0.1	0.05
4.	*Neolitsea cassia* (L.) Kosterm.	0.03	0.02
5.	*C. camphora* (L) J. Presl.	0.1	1.5

Analysis of essential oil samples was performed using GC/MS to evaluate differences in the oil composition. As depicted in fig. 1, essential oil of the bark of *C. sintoc, N. Cassia, C. burmanni, C. verum and C. champora* showed 23, 14, 4, 13 and 20 peaks, respectively. Oil from the leaves showed more peaks compared to that from the bark. The number of peaks separated from the oil of *C. sintoc, N. Cassia, C. burmanni, C. verum and C. champora* were 48, 74, 27, 35 and 18 respectively.

The components of essential oil studied are presented in table 2. Analysis of the oil components was based on retention time and a spectral search for the mass library. Data from GC/MS spectrometers were compared using a Databank instrument with WILEY 275 and the National Institute of Standards and Technology (NIST 3.0).

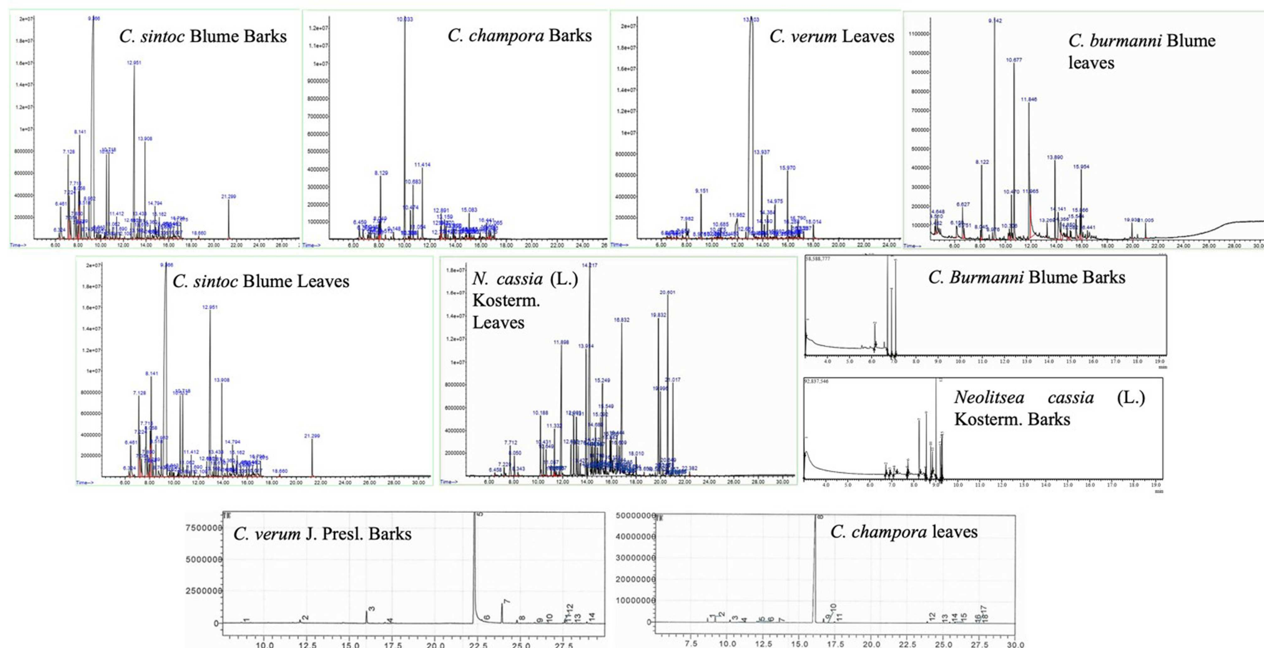

Fig. 1: GC chromatogram of Lauracea plants leaves and barks.

The library is equipped with a computer controlling the GC/MS. The relative amounts of each of the components were calculated based on the GC peak area. The databank of this device had been used to identify the presence of cinnamaldehyde, naphthalene, and copaena as major constituents of essential oil *C. burmannii* in several regions of Indonesia (Plumeriastuti *et al*., 2019).

The compounds identified from the bark of *C. burmanni* (Ness & Nees) Blume samples by comparison with the library consist of gamma-terpinene (29,30%), benzaldehyde (25,69%), 9-Octadecanoic acid, 1,2,3-propanetriyl (11,59%). The compounds identified from the leaf of *C. burmannii* cinnamaldehyde (25,24%), linalool (14,92%), alpha terpineol (12,65%), caryophyllene (4,77%). The compounds that have been reported in previous research through *Lauraceae* phytochemical analysis included trans-cinnamaldehyde (56.10%), 1,8-cineol (16.53%) and alpha-pinene (3.44%) (Chairunnisa *et al*., 2017). The bark of *Neolitsea cassia* consists of linalool (12.50%), beta-citronellol (15.39%), geraniol (11.02%) and citral (11.53%), The bark of *C. champora* consists of (+)-2-bornanone (32%), eucalyptol (8.54%), geraniol (8.03%) and alpha terpineol (8.09%). The leaf of *Neolitsea cassia* cinnamaldehyde (4.88%), eugenol (1.55%), caryophyllene (3.67%), caryophyllene oxide (2.13%) and 7-Fluoro-2H, 3H-[1,3]oxazolo[2,3-b]quinazolin-5-one (6.69%). The bark of *C. sintoc* consists of linalool (81.52%), naphthalene (2.62%), copaene (1.33%) and beta-caryophyllene (1.11%). The leaf of *C. sintoc* consists of linalool (41.56%), eugenol (5.52%) and caryophyllene (3.16%). The bark of *C. verum* consists of of eugenol (89.64%) and beta-caryophyllene (4.25%). The leaf of *C. verum* consists of 2-methoxy-3-(2-propenyl)- (77.32%). The bark of *C. champora* consists of (+)-2-Bornanone (32.05%), geraniol (8.03%), alpha-terpineol (80.38%), Eucalyptol (8.5%). The leaf of *C. champora* consists of the dominant compounds were camphor (91.93%), camphene (1.05%), endo-borneol (1.18%) and alpha terpineol. The content of beta-citronellol and e-citral in the bark of *Neolitsea cassia* which is, higher than the other four Lauraceae species, is responsible for its antibacterial properties.

Antibacterial activity by broth micro dilution

MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. As table 2 indicates, the oil from the barks appears to be less active than the leaf parts tested in this study. *Cinnamomum burmanni*, *C. verum* and *C. champora* have MIC and MBC values 6.25µg/mL, 12.5µg/mL, 3.12µg/mL, respectively. The smallest MIC is shown by *Neolitsea cassia* against SA and CA at 0.39µg/mL, 0.78µg/mL toward SE which could balance in inhibition of CA.

The oil from leaf parts was more active than the barks, where five samples showed high activity at the lowest concentration (0.39µg/mL). The highest activity was shown by *Neolitsea cassia* (L.) against two bacteria, followed by *C. burmanni* (Ness & Nees) Blume, *C. champora* and *C. verum.* The lowest activity of the left oil was given by *C. sintoc* with MIC higher than 3.12µg/mL, 1.25µg/mL and 1.56µg/mL.

In the case of *C. sintoc* bark oil, the highest inhibitory activity was observed against *Propionibacterium acnes*

Table 2: Result tested by microdilution method

Species	Part of species	Activity concentration (µg/mL)	Activity concentration *C. acnes*	Activity concentration *S. epidermidis*	Activity concentration *S. aureus*
C. sintoc Blume	B	MIC	<0.39	50	<12.5
		MB	<0.39	50	12.5
	L	MIC	3.12	12.5	1.56
		MBC	3.12	12.5	1.56
Neolitsea cassia (L.) Kosterm	B	MIC	0.39	0.78	1.56
		MBC	0.39	0.78	0.78
	L	MIC	<0.39	0.78	< 0.39
		MBC	<0.39	0.39	< 0.39
C. burmanni (Ness & Nees) Blume	B	MIC	3.12	25	50
		MBC	3.12	25	50
	L	MIC	3.12	6.25	< 0.39
		MBC	0.78	3.12	< 0.39
C. verum J. Presl.	B	MIC	6.25	< 0.39	< 0.39
		MBC	3.12	0.39	0.39
	L	MIC	1.56	<0.78	0.78
		MBC	0.78	<0.78	0.39
C. champora	B	MIC	12.5	12.5	6.25
		MBC	25	12.5	3.12
	L	MIC	< 0.39	1.56	1.56
		MBC	< 0.39	1.56	1.56

Note: B: Barks, L: Leaves, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

(Cutibacterium acnes) at concentrations <0.39µg/mL. Lesser activity toward *Staphylococcus aureus* (<12.5 µg/mL) and the least against *Staphylococcus epidermidis* (50µg/mL).

The bark of *C. burmanni* (Ness & Nees) Blume was less active in inhibiting bacteria than the previous samples against *Staphylococcus epidermidis* and *Staphylococcus aureus*, only 25µg/mL and 50µg/mL, except for *Cutibacterium acnes* 3.12µg/mL.

The bark of *C. verum* J. Presl and *Staphylococcus epidermidis* (<0.39 µg/mL) showed the inhibition balance against and Staphylococcus aureus (<0.39µg/mL), while Cutibacterium acnes was less active (6.25µg/mL). The bark of *C. champora* had the lowest activity against *Propionibacterium acnes (Cutibacterium acnes)* and *Staphylococcus epidermidis* (12.5µg/mL) while toward *Staphylococcus aureus*, it was at <0.39µg/mL. All stem barks showed good inhibitory activity against all bacteria with a MIC range of 0.39-50µg/mL.

DISCUSSION

The investigation into the anti-acne potential of various Lauraceae plants has yielded intriguing results, particularly highlighting the exceptional antibacterial activity of *Neolitsea cassia* leaves against both *Staphylococcus aureus* and *Cutibacterium acnes*. This finding not only expands our understanding of natural anti-acne agents but also opens new avenues for the development of plant-based acne treatments. The antibacterial activity test revealed a hierarchy of efficacy among the studied Lauraceae plants. Neolitsea cassia leaves demonstrated the highest activity, with a Minimum Inhibitory Concentration (MIC) of <0.39 ppm against both *S. aureus* and *C. acnes*. *Cinnamomum burmanni* and *C. verum* showed high activity (MIC <0.39 ppm) against *S. aureus* only, while *C. champora* exhibited high activity (MIC <0.39 ppm) against *C. acnes* only. This differential activity profile suggests a complex interplay between the plant compounds and bacterial species, indicating potential specificity in their mechanisms of action. The broad-spectrum activity of *N. cassia* against both tested bacteria is particularly noteworthy, as it suggests a potentially more versatile anti-acne agent compared to the other plants studied.

The GC-MS analysis revealed that *Neolitsea cassia* contains high concentrations of beta-citronellol, linalool, e-citral, and geraniol. These compounds were found in lower yields in the other plants studied, which correlates with the observed differences in antibacterial activity. This finding strongly suggests that the superior antibacterial efficacy of *N. cassia* is attributable to the presence and concentration of these specific compounds. Beta-citronellol has been previously reported to possess significant antimicrobial properties. A study by Herman et al. (2016) demonstrated its effectiveness against various Gram-positive and Gram-negative bacteria, including *S. aureus.* They proposed that beta-citronellol's mechanism of action involves disruption of bacterial cell membranes, leading to cell death. Linalool, a monoterpene alcohol,

has been extensively studied for its antimicrobial properties. Research by Zengin and Baysal (2014) showed that linalool exhibits strong antibacterial activity against S. aureus and other skin pathogens. They suggested that linalool's lipophilic nature allows it to penetrate bacterial cell membranes, causing structural and functional damage. E-citral, an isomer of citral, has demonstrated potent antibacterial activity in several studies. Work by Shi et al. (2017) revealed that citral effectively inhibits biofilm formation in S. aureus, which is particularly relevant in the context of acne treatment. The researchers proposed that citral interferes with quorum sensing mechanisms, thereby reducing bacterial virulence and biofilm formation. Geraniol, another monoterpenoid, has shown promising antibacterial properties. A study by Nowotarska *et al*. (2014) demonstrated that geraniol can disrupt bacterial cell membranes and inhibit essential cellular processes. The authors also noted synergistic effects when geraniol was combined with other antimicrobial agents, suggesting potential benefits in multi-compound formulations.

The exceptional activity of *N. cassia* leaves may not solely be due to the individual effects of these compounds but could also result from synergistic interactions between them. This synergy could potentially explain why *N. cassia* outperforms the other plants, even though they contain some of the same compounds in lower concentrations. Furthermore, the differential activity observed against *S. aureus* and *C. acnes* across the plant species suggests that additional, unidentified compounds or specific ratios of the identified compounds may play crucial roles in determining antibacterial specificity. This observation opens up new research directions for exploring novel antibacterial mechanisms and compound interactions.

CONCLUSION

All bark and leaves of five Lauraceae plants contain essential oils. The highest essential oil content was found in the leaves of *C. champora*, namely 1.5%, while the barks the bark of *C. burmanni, C. verum* and *C. champora* 0.1%, respectively. The essential oil that has the greatest activity against disease microbes was Neolitsea cassia oil with MIC for CA and *Staphylococcus aureus* < 0.39. The volatile oil components in the five stem bark and leaves of the five plants studied were different even though they were all from the same tribe, namely Lauraceae.

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